

**AMENDMENTS TO THE SPECIFICATION**

**Please replace the paragraph beginning at page 6, line 15 with the following:**

Fig. 5 is a graph showing the time course of FRET response of Fflip-pm in CHO cells (ratio of degree of CFP ( $480 \pm 15$  nm) excited at  $25^{\circ}\text{C}$  and  $440 \pm 10$  nm to emission intensity of YFP ( $535 \pm 12.5$  nm) in the Example of this invention (Each arrow means addition of  $\text{PIP}_3$  ( $1\text{--}10 \mu\text{M}$ )).

**Please add the following paragraph at page 8, after line 9:**

Fig. 15 is a graph showing the time course of CFP/YEF emission intensity ratio when PMA was added to DAG-flip-pm and DAG-flip-em in the Example of this invention.

**Please replace the paragraph beginning at page 11, line 5 with the following:**

Probe *1* of this invention has a membrane localization sequence *5* at the terminal thereof for tethering itself at a membrane, since lipid second messenger *6* is produced in membrane *8* such as plasma membranes and endomembranes. Such membrane localization sequence *5* is linked to any of the chromophores and has a role of tethering probe *1* at membrane *8*. To be more specific, for tethering the probe to cell membranes, a lipidizatoin sequence such as K-Ras and N-Ras (Resh, M. D. (1996) *Cell. Signal.*, 8, 403-412) and transmembrane sequence are exemplified. By appropriately selecting membrane localization sequence *5* depending upon lipid second messenger *6* to be detected or membrane *8* to be tethered, probe *1* is able to be tethered not only to plasma membranes or endomembranes but also to other organelle membranes such as inner membrane of nucleus or outer membrane of mitochondria. To be more specific, C181S variant of N-Ras and C181 variant-eNOS of N-Ras for endoplasmic reticulum membrane and Golgi body membrane; Tom20 for mitochondrial membrane; caveolin for caveola; and Cbp for



raft may be exemplified. In addition, lipid second messenger **6** on organelle membrane such as other nuclear membrane or peroxisome membrane may be detected by using a localization sequence of a protein localized in each organelle membrane.

**Please replace the paragraph at page 17, line 1 with the following:**

Introduction of the probe (~~CGY~~) into CHO-PDGFR cells

**Please replace the paragraph beginning at page 18, line 20 with the following:**

It was confirmed from Fig. 5 that the CFP:YFP emission ratio of Fllip-pm rapidly decreased by addition of synthetic PIP<sub>3</sub> (1-~~1~~ μM) and reached a plateau. Therefore, it was noted that FRET from CFP to YFP dependently increased on PIP<sub>3</sub>, and Fllip-pm could be used for visualizing PIP<sub>3</sub> dynamic on plasma membrane.